adult mast cells of the mesentery of rats after moderate intraperitoneal doses of the histamine liberator, compound 48/80. After larger doses, which cause disintegration of mesenteric mast cells, mitoses are found in mast cells of the subcutaneous tissue. 2) Replacement of cells after disintegration appears to be by heteroplastic differentiation of lymphocytes or undifferentiated mesenchymal cells or both.

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Co^{60} Vitamin B_{12} Binding Capacity of Normal Human Serum.* (22888)

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Recent studies(1-4) of plasma concentration and transport mechanisms of vit. B_{12} have included microbiological measurements of its serum *in vitro* binding capacity. Results have varied widely. The present investigations are concerned with estimation of Co^{60} vit. B_{12} serum binding capacity.

Methods. Sera were obtained from 40 normal persons. The Co^{60} vit. B_{12} had a specific activity of 0.786 mc/mg. Its physiological activity was proved when 3 μg given intravenously to a patient with pernicious anemia in relapse induced a moderate reticulocyte response and clinical improvement. To 1 ml of serum was added an aliquot of Co^{60} vit. B_{12} ranging from 1 to 500 μg. This was done either by adding the chosen amount of vitamin directly, or by making an initial concentrated solution in serum, then rediluting with more serum at once. These methods gave equal results. Specimens were prepared in duplicate, one to serve as a standard. Each was pipetted into a Visking bag. Samples were incubated at room temperature for 2 hours, then subjected to constant agitation and exhaustive dialysis in cold, running tap water for 48 hours. Standards were placed in 4 ml vials at once; samples were placed in similar vials at conclusion of dialysis. Each vial was filled with concentrated sulfuric acid, dissolving the bag and allowing complete mixing within the vial. Counting was done in a well-type scintillation counter. "Bound" vit. B_{12} was calculated as activity retained in the bag after dialysis. Parallel studies were made with $Co^{60}Cl_2$ and sera from 14 normal subjects. The specific activity and cobalt content of the $Co^{60}Cl_2$ solution were equal to that of the radioactive vit. B_{12}. Continuing dialysis for 72 or 96 hours did not decrease residual activity further than the 48-hour period used. Incubation of the serum-B_{12} mixture for more than 2 hours prior to dialysis did not alter amount of vit. B_{12} bound. Dialysis for 48 hours in a reservoir of normal saline, 6% dextran in normal saline, or human plasma 50x the volume of the samples and changed at 24 hours gave the same results as exhaustive dialysis in tap water. Co^{60} B_{12} in saline dialyzed for 48 hours in tap water showed residual activity within the Visking bag of less than 2% at any concentra-

*Co^{60} Vit. B_{12} was obtained from Merck & Co, Rahway, N. J.

TABLE I. Co^{60} Vitamin B_{12} Binding Capacity of Normal Human Serum.

mμg $Co^{60}B_{12}$ added per ml serum	No. of sera tested	% $Co^{60}B_{12}$ bound ± 1 S.D.	mμg $Co^{60}B_{12}$ bound	Serum protein, g %	mμg $Co^{60}B_{12}$ bound per g serum protein
1	23	88.5 ± 13.1	0.89	6.9	12.8
2.5	23	67.4 ± 6.3	1.69	6.9	24.4
5	23	42.9 ± 3.9	2.15	6.9	31.1
10	10	34.0 ± 5.2	3.40	6.7	50
25	10	24.3 ± 4.8	6.08	6.7	101
50	10	22.7 ± 2.4	11.35	6.7	168
100	7	26.3 ± 3.2	26.3	7.5	352
250	7	26.3 ± 4.8	66.6	7.5	878
500	7	26.0 ± 2.5	130	7.5	1743

TABLE II. $Co^{60}Cl_2$ Binding Capacity of Normal Human Serum.

Amt $Co^{60}Cl_2$* added per ml serum	No. of sera tested	% $Co^{60}Cl_2$ bound	Amt $Co^{60}Cl_2$ bound
1	6	84.2	0.84
2.5	6	88.0	2.2
5	6	85.8	4.3
10	2	89.0	8.9
25	2	90.0	22.5
50	2	85.5	42.7
100	5	76.0	76

* Contains Co in equal amounts to corresponding aliquots of $Co^{60}B_{12}$.

tion. When the bag was opened and washed following dialysis of the B_{12}-serum, activity was found on the casing ranging from 8% of the total residual activity at the lowest concentration of vitamin to 3% at the highest. No correction was made for this activity.

Results. Table I shows that as the vit. B_{12} aliquot was increased, the fraction bound fell to a plateau while the absolute amount rose. The highest amount bound was 130 μg/ml serum. The standard deviation at each level showed a narrow range of individual binding capacities. Calculated binding capacity per gram of serum protein paralleled that of whole serum.

Analogous studies on serum binding of $Co^{60}Cl_2$ are shown in Table II. A high percentage is bound at all concentrations. These data are shown graphically in Fig. 1 and 2.

Discussion. Bound vit. B_{12} is defined by microbiological assay as that portion of the vitamin which becomes available to the organism only after the B_{12}-serum is heated to 100°C for 30 minutes. Rosenthal(1), using *Lactobacillus leichmannii* for assay after dialyzing the B_{12}-serum 48 hours, showed bindings in mixtures of 2 and 6 mμg of vit. B_{12} per ml serum similar to those in the present study. However, when as much as 50 mμg were added per ml, no more than 3 mμg were bound. Pitney(2), using *Euglena gracilis,* reported average binding of 178 μμg/ml after adding 1,000 μμg, and noted that when more than 500 μμg were added per ml, most of the vitamin was unbound. Lear(3) reported similar findings with *E. gracilis*. Mollin(4) found that adding 20,000 μμg per ml serum resulted in binding of only 225 μμg. The above results with Co^{60} vit. B_{12} show a much higher binding capacity than any of the preceding reports. That this is not due to ionic

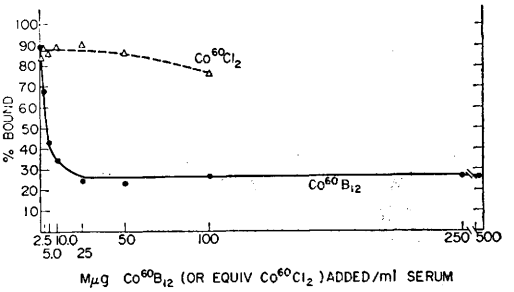

FIG. 1.

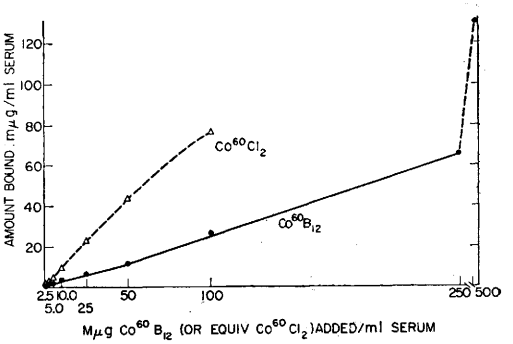

FIG. 2.

cobalt from possible breakdown of the vit. B_{12} molecule is suggested by the different binding capacity of serum for $Co^{60}Cl_2$.

Pitney(2) found vit. B_{12} bound to an alpha globulin *in vivo.* However, when the vitamin was added to serum *in vitro,* some travelled electrophoretically with beta globulin. This fraction of vitamin, unlike that bound to alpha globulin, was directly available to the test organism, and was consequently termed "free." The discrepancy in binding capacities assayed by the different methods of measurements may, therefore, occur because the amount of vit. B_{12} made available to a micro-organism by heating serum does not represent the total vitamin bound to the serum proteins. It is possible that though the vitamin is bound to an alpha globulin at usual body levels, this mechanism is quickly saturated and nonspecific binding to other serum protein fractions takes place at higher concentrations.

Summary. A method is presented for estimating binding capacity of serum for radioactive vit. B_{12}. This capacity was found to increase as the concentration of the vitamin was raised. "Bound" vit. B_{12} measured in this way is not synonymous with that considered bound because it is unavailable to certain microorganisms.

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Effect of Reserpine (Serpasil®)* on Oxygen Consumption of Euthyroid, Hypothyroid, and Hyperthyroid Guinea Pigs. (22889)

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Experimental observations regarding the influence of reserpine on thyroid function are contradictory. Although reserpine completely antagonized increase in oxygen consumption produced by exogenous thyroxine in rats(1), it produced neither significant change in metabolic rate(2) nor consistent alteration in 24-hour I^{131} uptake of thyroid gland of euthyroid rats(3). However, reserpine exhibits a significant direct antithyroid effect *in vitro,* an effect which consisted chiefly of inhibition of organic binding of I^{131}(4). Patients placed on prolonged reserpine therapy showed no change in thyroid status(3) or metabolic rate(5), and Ford, *et al.*(6) were unable to demonstrate any change in basal metabolic rate or I^{131} uptake of the thyroid gland in euthyroid patients after chronic treatment with total alkaloid extract of *Rauwolfia serpentina.* Moncke(7), however, found that chronic reserpine therapy over a period of months produced "normalization" of basal metabolic rate in a group of 15 hyperthyroid patients and was more effective than methyl thiouracil in combating cardiac arrythmias associated with the disease. When reserpine was used in combination with methyl thiouracil, smaller doses of both drugs were needed. Most studies have been concerned chiefly with the effect of reserpine in a single thyroid state.

To resolve the apparent dissimilarities in response to this alkaloid, experiments were conducted to compare its effects on oxygen consumption of hypothyroid, euthyroid, and hyperthyroid guinea pigs.

Materials and methods. A modification of

* Serpasil used was generously supplied by Ciba Pharmaceutical Products Inc., Summit, N. J.

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